RIBOSOMAL SUBUNIT EXCHANGE IN *DICTYOSTELIUM PURPUREUM*

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INTRODUCTION

The rapid and frequent exchange of the 70S ribosomes in *Escherichia coli*, with their 50S and 30S subunits, has been established by kinetic studies and heavy isotope techniques (1–4). While these studies were in progress, it was reported that ribosomes of *Candida krusei*, a eucaryote, undergo frequent exchange of ribosomal subunits (5). It has been suggested that ribosomes dissociate and re-form between successive rounds of protein synthesis.

The mechanism of polypeptide initiation in procaryotes has been well documented (6–8). On the other hand, still very little is known about protein initiation in eucaryotic organisms. Early works on ribosomal synthesis in HeLa cells suggested that ribosomal subunits participate in polyribosome formation prior to being assembled in single monosomes (9, 10). It has also been shown that vaccinia-virus RNA becomes associated with the smaller ribosomal subunit before appearing on polysomes (11).

The cellular slime molds have been mainly studied as a model organism for problems concerning morphogenesis and differentiation (12, 13). The life cycle can be easily subdivided into a phase of growth (cell division) and differentiation. During the growth phase, the amebas feed on bacteria and divide by mitosis approximately every 3 hr. These organisms thus offer great advantages to study ribosome dissociation and re-assembly by both kinetics and heavy isotope techniques.

MATERIALS AND METHODS

Since the amebas of *Dictyostelium purpureum* have not been shown to grow on a defined medium, they cannot be grown directly on heavy isotopes. To circumvent this difficulty, *E. coli* B/r wild type was grown first on the following minimal media: K_2HPO_4, 1.2 g; KH_2PO_4, 0.5 g; Na_3 citrate $5H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $N^{15}H_4Cl$, 1.0 g; 0.3% glucose, 1 liter of D_2O. The bacteria were then suspended and washed twice with 0.016 M Sorensen's buffer, pH 6.0, and re-suspended in the same buffer at 5×10^9 cells/ml. Spores of *D. purpureum* were inoculated at 10^4 spores/ml, and growth was recorded with a Zeiss chamber (Carl Zeiss, Inc., New York). When the amebas are grown on heavy bacteria, the cells divide approximately every 6 hr.

RESULTS AND DISCUSSION

Before demonstrating that ribosomal subunits undergo exchange during the cell cycle, it was necessary to show that the subunits are stable during the mitotic cycle. Slime mold amebas were grown on heavy bacteria in the presence of 10 μCi/ml of uridine-^{3}H (specific activity 26.5 Ci/mmole), until the cells had reached a concentration of approximately 5×10^5 cells/ml. The amebas were then washed several times with Sorensen's buffer at room temperature and transferred to medium containing light bacteria in the presence of 2.5 μCi/ml of amino acids-^{14}C (specific activity, 52 mCi/m atom of carbon) and allowed to grow for 1.5–2.0 generations. The cells were harvested, washed free of bacteria, and lysed with 0.5% deoxycholate (DOC) in Tris-HCl buffer (14), in the presence of a 5- to 10-fold excess of cold-carrier amebas. The lysate was analyzed with sucrose linear gradients (for details see legend to Fig. 1).

Fig. 1 shows clearly that the two ribosomal subunits are stable during cell replication. The subunits synthesized while the cells were grown on light bacteria (^{14}C) sediment in the region of the subunits derived from the cold-carrier cells. On the other hand, the ribosomal subunits synthesized when the cells were growing on heavy bacteria (^{3}H) sediment faster and the monodisperse peak in each case indicates no exchange or lack of stability between the two populations of subunits. Note further that the light single-monosomes (^{14}C) are separated from the heavy and/or hybrid monosomes (^{3}H).

If the single monosomes of *D. purpureum* undergo dissociation and reassociation during the cell cycle, it should be possible to find hybrid monosomes when cells are shifted from heavy to light bacteria. Amebas were grown for at least six

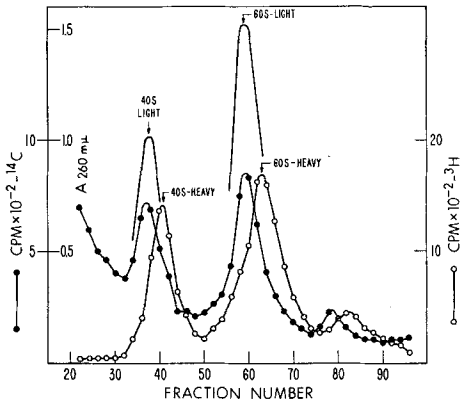

FIGURE 1 The DOC lysate was centrifuged at 15,000 rpm for 20 min to remove the heavy polysomes and cell debris, the supernatant was layered over a 5–20% linear sucrose gradient, and sedimentation was carried out for 6 hr at 22,000 rpm, SW 25, at 0°C. Each gradient was collected with an Isco fraction-collector (Instrumentation Specialties Co., Lincoln, Neb.) at a flow rate of 2 ml/min in 0.25 ml fractions. Each fraction was counted in 10 ml of Bray's solution. Those fractions containing the light 60S and 40S subunits, from the carrier amebas, were defined by OD 260 mμ (solid line), A 260 mμ; open circle), ^{3}H, cells grown on heavy bacteria; (closed circle), ^{14}C, heavy amebas transferred to light bacteria. Note on the right side of the gradient that some separation was obtained between the light and heavy and/or hybrid monosomes.

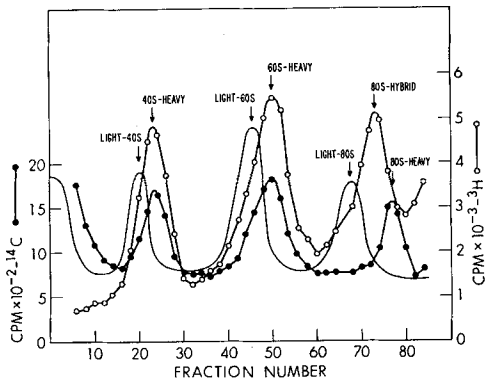

FIGURE 2 The experiment was carried out as described in the text. The hyperbolic gradient was constructed by mixing 8% and 20% sucrose in the appropriate buffers. The gradient was centrifuged for 7.5 hr at 24,000 rpm, SW 25, at 0°C. 0.25 ml fractions were collected and counted in Bray's solution. (Solid line), A 260 mμ; (open circle), ^{3}H, cells grown on heavy bacteria and then transferred to light bacteria without isotope; (closed circle), ^{14}C, amebas grown constantly on heavy bacteria.

generations in the presence of heavy bacteria and uridine-^{3}H. When the cells had reached 10^{6} amebas/ml, they were transferred to light bacteria and allowed to grow for two to three generations without isotope. Concomitantly, amebas were also grown on heavy bacteria in the presence of amino acids-^{14}C. At the end of the established growth period, the two populations were mixed together along with cold-carrier cells. The mixture was lysed with 0.5% DOC, as described, but at a Mg^{2+} concentration sufficient to prevent dissociation of all the monosomes (8 mM, Mg^{2+}) (14). The results are reported in Fig. 2. Again, we find that the ribosomal subunits are stable, since even after going through two to three cell cycles, in light bacteria, the radioactivity (^{3}H) sediments as a sharp peak in the same region as in the case of amebas that had been constantly grown on heavy bacteria (^{14}C). The gradient also distinguishes between the heavy monosomes, the hybrid monosomes, and the light monosomes from the carrier cells, indicating that random exchange of the ribosomal subunits must have taken place during the cell cycle.

Other types of experiments were also done. Purified heavy, light, and hybrid monosomes were mixed and centrifuged in sucrose gradients. In all cases we were always able to separate the three types.

Kaempfer et al. (3) were able to separate the two types of hybrid monosomes according to density by CsCl gradients. By this method, monosomes which contained the 50S (heavy) and 30S (light) subunits could be distinguished from those composed of the 50S (light) and 30 S (heavy) subunits. This method failed to separate the two types of hybrid monosomes in slime mold amebas, but was able to distinguish the heavy, hybrid and, light monosomes (Fig. 3). Our lack of resolution may be due to the fact that insufficient ^{15}N was incorporated into the ribosomal RNA (rRNA) to give enough specific-density difference between the two hybrids.

The present study supports the thesis that monosomes of eucaryotic cells undergo exchange during cell proliferation (5). But whether or not this exchange is obligatory for protein initiation and synthesis cannot at the moment be satisfactorily answered. The data available from eucaryotic systems suggest that the single monosomes do not dissociate when released from polyribosomes

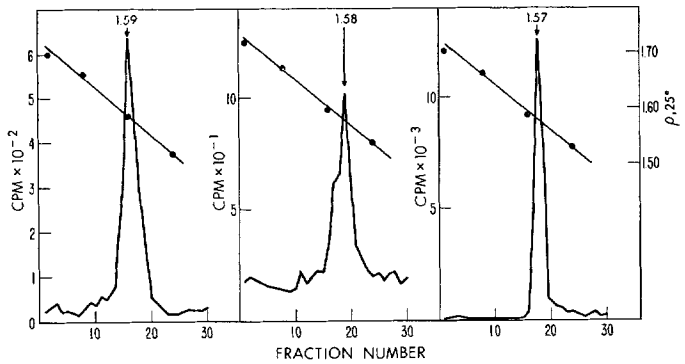

FIGURE 3 CsCl density-gradients of partially purified monosomes. Amebas were growing in three different ways: (*a*) growth on heavy bacteria for at least five generations, in the presence of uracil-^{14}C; (*b*) growth on heavy bacteria in the presence of uridine-^{3}H, then transferred to light bacteria and grown for a couple of generations without isotopes; and (*c*) growth on light bacteria in the presence of uracil-^{14}C. The amebas were harvested as described and lysed with Nonidet P40, centrifuged at 15,000 rpm for 15 min. The supernatant was layered over a 5–20% linear sucrose-gradient and centrifuged for 5 hr at 25,000 rpm, SW 25, at 0°C. The gradients were collected in 0.5 ml fractions, the radioactivity corresponding to the monosomes was fixed in 3% glutaraldehyde, and a portion was placed on top of a preformed CsCl gradient (16), and centrifuged for 12 hr at 37,500 rpm, SW 39 at 2°C. The gradients were collected in 0.15 ml fractions, coprecipitated in the presence of serum albumin with 10% TCA, placed on Millipore filters (Millipore Corp., Bedford, Mass.), and counted in a liquid scintillation counter. On the left side, the heavy monosomes (1.59); on the right, the light (1.57); and in the middle, the hybrids (1.58).

(15), and thus it is possible that they may be reused without going through a dissociation cycle.

SUMMARY

By the use of heavy isotope techniques, data have been obtained and are presented which support the thesis that monosomes of eucaryotic cells undergo exchange during cell proliferation (5). The data further show that the ribosomal subunits are stable and that the exchange occurs at random.

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